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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: GERONI Cristina et al.
SERIAL NO.: NEW U.S. PCT APPLICATION
FILED: HEREWITH
INTERNATIONAL APPLICATION NO.: PCT/EP00/00745
INTERNATIONAL FILING DATE: January 31, 2000
FOR: ANTITUMOUR SYNERGISTIC COMPOSITION

REQUEST FOR PRIORITY UNDER 35 U.S.C. 119 AND THE INTERNATIONAL CONVENTION

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In the matter of the above-identified application for patent, notice is hereby given that the applicant claims as priority:

COUNTRY	APPLICATION NO	DAY/MONTH/YEAR
Great Britain	9904387.9	25 February 1999

Certified copies of the corresponding Convention application(s) were submitted to the International Bureau in PCT Application No. PCT/EP00/00745. Receipt of the certified copy(s) by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.

Respectfully submitted,
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Signed Andrew Gerry

Dated 13 October 1999

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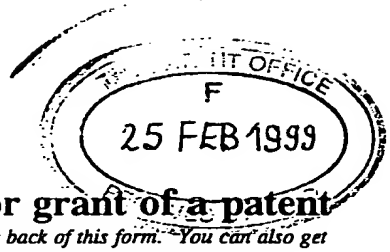

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Request for grant of a patent

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The Patent Office

Cardiff Road
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1. Your reference — P.76286 GCW.CMK

2. Patent application number *(The Patent Office will fill in this part)* — **9904387.9** — 25 FEB 1999

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

PHARMACIA & UPJOHN S.P.A.

Via Robert Koch 1.2
20152 Milan
Italy

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — Italy 10000100

4. Title of the invention — ANTITUMOUR SYNERGISTIC COMPOSITION

5. Name of your agent *(if you have one)* — J A KEMP & CO

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

14 SOUTH SQUARE
GRAY'S INN
LONDON WC1R 5LX

Patents ADP number *(if you know it)* — 26001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer "Yes" if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body:
See note (d))

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Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	6
Claim(s)	2
Abstract	1
Drawing(s)	

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Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	2 x 5
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application

Signature JAKemp & 6 Date 25 February 1999

12. Name and daytime telephone number of person to contact in the United Kingdom: Mrs C M Keen 0171 405 3292

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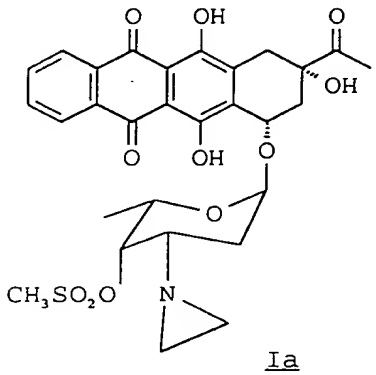
Antitumor Synergistic Composition

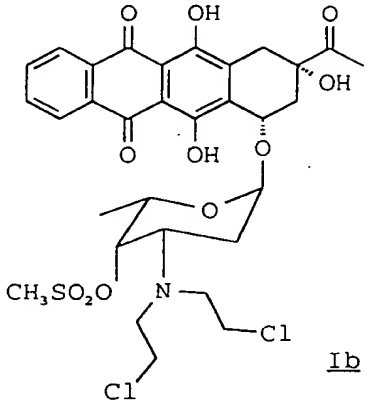
The present invention relates in general to the field of cancer treatment and, more particularly, provides an antitumor composition comprising an alkylating anthracycline and a topoisomerase II inhibitor, having a synergistic or additive antineoplastic effect.

The present invention provides, in a first aspect, a pharmaceutical composition for use in antineoplastic therapy in mammals, including humans, comprising

- an alkylating anthracycline of formula Ia or Ib :



Ia



Ib

- an antineoplastic topoisomerase II inhibitor, and a pharmaceutically acceptable carrier or excipient.

The chemical names of the alkylating anthracyclines of formula Ia and Ib are 4-demethoxy-3'-deamino-3'-aziridinyl-4'-methansulfonyl daunorubicin (Ia) and 4-demethoxy-N,N-bis(2-chloroethyl)-4'-methansulfonyl daunorubicin (Ib). These alkylating anthracyclines were described in Anticancer Drug Design (1995), vol. 10, 641-653, and claimed respectively in US-A-5,532,218 and US-A-5,496,800. Both compounds intercalate into DNA via the chromophore and alkylate guanine at N^7 position in DNA minor groove via their reactive moiety on position 3' of the amino sugar. Compounds Ia and Ib are able to circumvent the resistance to all major classes of

cytotoxics, indicating that the compounds represent a new class of cytotoxic antitumor drugs.

Topoisomerase II inhibitors are described in various scientific publications. The main representatives of this wide class of drugs are: the anthracycline derivatives such as doxorubicin, daunorubicin, epirubicin, nemorubicin and idarubicin; the podophyllotoxin compounds etoposide and teniposide; the anthraquinone derivative like mitoxantrone and amsacrine. See for example the review: Cancer, Principles and Practice of Oncology, Lippincott-Raven Ed. (1997), 452-467. Doxorubicin and etoposide are the preferred topoisomerase II inhibitors to be used in the present invention. The present invention also provides a product comprising an alkylating anthracycline of formula Ia or Ib as defined above and an antineoplastic topoisomerase II inhibitor, as combined preparation for simultaneous, separate or sequential use in antitumor therapy.

A further aspect of the present invention is to provide a method of treating a mammal including humans, suffering from a neoplastic disease state comprising administering to said mammal an alkylating anthracycline of formula Ia or Ib as defined above and an antineoplastic topoisomerase II inhibitor, in amounts effective to produce a synergistic antineoplastic effect.

The present invention also provides a method for lowering the side effects caused by antineoplastic therapy with an antineoplastic agent in mammals, including humans, in need thereof, the method comprising administering to said mammal a combination preparation comprising an antineoplastic topoisomerase II inhibitor as defined above and an alkylating anthracycline of formula Ia or Ib, as defined above, in

amounts effective to produce a synergistic antineoplastic effect.

By the term "a synergistic antineoplastic effect" as used herein is meant the inhibition of the growth tumor, preferably the complete regression of the tumor, administering an effective amount of the combination of an alkylating anthracycline of formula Ia or Ib as defined above and a topoisomerase II inhibitor to mammals, including human.

By the term "administered " or "administering" as used herein is meant parenteral and /or oral administration. By "parenteral" is meant intravenous, subcutaneus and intramuscolar administration. In the method of the subject invention, the alkylating anthracycline may be administered simultaneously with the compound with the topoisomerase II inhibitor activity, for example of the anthracycline or etoposide class, or the compounds may be administered sequentially, in either order. It will be appreciated that the actual preferred method and order of administration will vary according to, inter alia, the particular formulation of the alkylating anthracycline of formula Ia or Ib being utilized, the particular formulation of the topoisomerase II inhibitor, such as one of the anthracycline or etoposide class, being utilized, the particular tumor model being treated, and the particular host being treated.

In the method of the subject invention, for the administration of the alkylating anthracycline of formula Ia or Ib, the course of therapy generally employed is from about 0.1 to about 200 mg/m^2 of body surface area. More preferably, the course therapy employed is from about 1 to about 50 mg/m^2 of body surface area.

In the method of the subject invention, for the administration of the topoisomerase II inhibitor the course of therapy

generally employed is from about 1 to about 1000 mg/m^2 of body surface area. More preferably, the course therapy employed is from about 10 to about 500 mg/m^2 of body surface area. The antineoplastic therapy of the present invention is in particular suitable for treating breast, ovary lung, colon, kidney, stomach, pancreas, liver, melanoma, leukemia and brain tumors in mammals, including humans.

In a further aspect, the present invention is directed to the preparation of a pharmaceutical composition containing an effective amount of an alkylating anthracycline of formula Ia or Ib as defined above and an antineoplastic topoisomerase II inhibitor in the prevention or treatment of metastasis or for the treatment of tumors by angiogenesis inhibition, as well as to the use of an alkylating anthracycline of formula Ia or Ib as defined above and an antineoplastic topoisomerase II inhibitor for the treatment of tumors by angiogenesis inhibition or for the treatment or prevention of metastasis .

As stated above, the effect of an alkylating anthracycline of formula Ia or Ib and a topoisomerase II inhibitor, such as an anthracycline or etoposide derivative, is significantly increased without a parallel increased toxicity. In other words, the combined therapy of the present invention enhances the antitumoral effects of the alkylating anthracycline and of the topoisomerase II inhibitor and thus yields the most effective and least toxic treatment for tumors. The superadditive actions of the combination preparation of the present invention are shown for instance by the following *in vivo* tests, which are intended to illustrate but not to limit the present invention.

Table 1 shows the antileukemic activity on disseminated L1210 murine leukemia obtained combining Ia with etoposide. At the dose of 30 mg/kg of etoposide alone (day +3) and at the dose

of 1 mg/kg of Ia alone (days +1,2) were associated, without toxicity, with ILS% values of 100 and 67, respectively. Combining etoposide and Ia at the same doses with the same schedule an increase of activity with ILS% values of 450 was observed, indicating a synergistic effect.

Table 2 shows the antileukemic activity on disseminated L1210 murine leukemia obtained combining Ia with doxorubicin. At the dose of 13 mg/kg of doxorubicin alone (day +3) and at the dose of 1.5 mg/kg of Ia alone (days +1,2) were associated, without toxicity, with ILS% values of 50 and 67, respectively. Combining doxorubicin and Ia at the same doses with the same schedule an increase of activity with ILS% values of 150 was observed, indicating a synergistic effect.

For these experiments Ia was solubilized in [Cremophor® /EtOH= 6.5:3.5]/[normal saline]=20/80 v/v, while standard etoposide pharmaceutical preparation and doxorubicin solubilized in water were used.

Table 1: Antileukemic activity against disseminated L1210[1] murine leukemia of Ia in combination with Etoposide

Compound	Treatment schedule	Dose[2] (mg/kg/day)	ILS%[3]	Tox[4]	LTS[5]
Ia	iv +1,2	1	67	0/10	0/10
Etoposide	iv +3	30	100	0/10	0/10
Ia + Etoposide	iv +1,2 iv +3	1 + 30	450	0/10	4/10

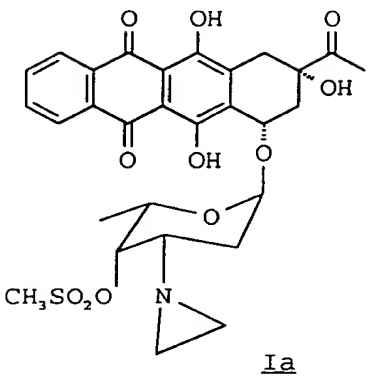
Table 2: Antileukemic activity against disseminated L1210[1] murine leukemia of Ia in combination with Doxorubicin

Compound	Treatment schedule	Dose[2] (mg/kg/day)	ILS%[3]	Tox[4]	LTS[5]
Ia	iv +1,2	1.5	67	0/10	0/10
Doxorubicin	iv +3	13	50	0/10	0/10
Ia + Doxorubicin	iv +1,2 iv +3	1.5 + 13	150	0/10	3/10

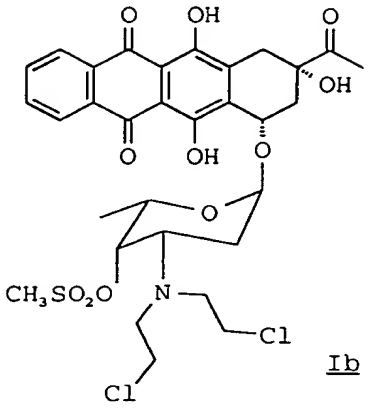
1) L1210 leukemia cells (10^5/mouse) are injected iv on day 0.
2) Treatment is given starting on day 1 after tumor transplantation (day 0).
3) Increase in life span: [(median survival time of treated mice/median survival time of controls) x 100]-100
4) Number of toxic deaths/number of mice.
5) Long Term Survivors (>60 days) at the end the experiment.

Claims

1. Products containing an alkylating anthracycline of formula Ia or Ib:



Ia



Ib

and an antineoplastic topoisomerase II inhibitor as a combined preparation for simultaneous, separate or sequential use in the treatment of tumors.

2. Products according to claim 1 wherein the alkylating anthracycline is 4-demethoxy-3'-deamino-3'-aziridinyl-4'-methansulfonyl daunorubicin.

3. Products according to claim 1 or 2 wherein the topoisomerase II inhibitor is etoposide.

4. Products according to claim 1 or 2 wherein the topoisomerase II inhibitor is doxorubicin.

5. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or excipient and, as active ingredient, an alkylating anthracycline of formula Ia or Ib as defined in claim 1 and an antineoplastic topoisomerase II inhibitor.

6. A composition according to claim 5 wherein the topoisomerase II inhibitor is doxorubicin or etoposide.

7. Use of an alkylating anthracycline of formula Ia or Ib as defined in claim 1 and an antineoplastic topoisomerase II

inhibitor in the preparation of a medicament for use in the treatment of tumors.

8. Use according to claim 7 wherein the topoisomerase II inhibitor is etoposide or doxorubicin.

9. Use of an alkylating anthracycline of formula Ia or Ib as defined in claim 1 and an antineoplastic topoisomerase II inhibitor in the preparation of a medicament for use in the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis.

ABSTRACT

Antitumor Synergetic Composition

There are provided the combined use of 4-demethoxy-3'-deamino-3'-aziridinyl-4'-methansulfonyl daunorubicin or 4-demethoxy-N,N-bis(2-chloroethyl)-4'-methansulfonyl daunorubicin and an antineoplastic topoisomerase II inhibitor in the treatment of tumors and the use of said combination in the treatment or prevention of metastasis or in the treatment of tumors by inhibition of angiogenesis.